ISSN 0704-3716

Canadian Translation of Fisheries and Aquatic Sciences

No. 5443

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Original title: Etude électrophysiologique des réactions de la grenouille au courant électrique continu

In: Ann. Hydrobiol. 2(1), 1971, p. 5-23

Original language: French

Available from:
Canada Institute for Scientific and Technical Information
National Research Council
Ottawa, Ontario, Canada K1A 0S2

1989

34 typescript pages

Ann. Hydrobiol., 1971, 2 (I), 5-23.

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Annales d'Hydrobiologie
National Agronomic Research Institute
149, rue de Grenelle, Paris 7e

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Abstract

Frogs were placed in a uniform electric field. Their reactions in relation to their orientation with respect to the flow of the current and current intensity are described.

The origin of each of these reactions is demonstrated (excitation of muscle or nerve fibers; increase or decrease in motoneuron excitability).

The method and results are discussed in terms of their applications to other aquatic species, notably fish.

Introduction

Most aquatic animals react to the passage of direct current in the liquid environment surrounding them. The effects are seen primarily as motor phenomena, i.e., intense tetanic contractions or else, paralysis. The effect may be more subtile in fish and result in swimming patterns that systematically guide the fish to the same electrode. This 'imposed' swimming pattern has proved effective enough to have been used as a means of catching fish.

The practical value of this type of fishing plus improvements in the equipment used have made it desirable to acquire a better understanding of

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the effects of current. A certain number of studies have already been carried out to this end, but in most of them, the approach has consisted only in sectioning and destroying certain parts of the nervous system with the result that conclusions have been indirect and often hypothetical (Blancheteau et al., 1961). The present authors considered that with modern electrophysiological techniques, a more direct study of current action on the nerve and muscle structures involved would be possible. This is the approach we adopted.

Before undertaking an electrophysiological study of the specific reactions of fish to electric current, we wished to solve a certain number of more general problems by investigating the effect of current in an animal in which the nerve and muscle structures could be more easily isolated. For this reason, we chose the frog as our experimental animal.

A table was drawn up of the various reactions to direct current in frogs. Some of these reactions have already been described; namely, by Köllensperger and Scheminzky (1938). We have tried to provide a functional classification.

In addition, muscle and nerve-muscle preparations isolated from the same animal were subjected to direct current under physical conditions that were as close as possible to those existing for the tissues in intact animals and the resulting reactions were recorded.

Analysis of both sets of observations enabled us to explain most of the reactions to direct current encountered in frogs.

1. - DESCRIPTION OF REACTIONS

When a frog is placed in a liquid medium traversed by direct current, it usually responds by what seems to be fairly ill-defined muscle activity. It quickly becomes clear that the animal's reaction depends on its orientation in relation to the flow of current and on the intensity of the current. By systematically modifying these parameters, a certain number of typical and reproducible reactions can be defined. This was accomplished by maintaining the animal's body successively in each of the following three selected positions:

- parallel to the flow of the current, with the head facing the cathode;
- parallel to the flow of the current, with the head facing the anode;
- perpendicular to the flow of the current.

1.1. Experimental Procedure

Frog specimens of the species Rana esculenta, weighing an average of 60 to 70 g each, were placed in a plastic, rectangular tank, 120 cm X 30 cm, with 10 cm of water. The electrical resistivity of the latter was roughly 3 000 ohm-cm.

The short sides of the tank were covered entirely with two stainless steel plates acting as electrodes. This made it possible to establish a perfectly uniform electric field.

The direct current (0 to 150 V) was supplied by a source stabilized to within 0.05 %. The potential gradient (V/cm) was calculated from the potential difference between the extremities of the tank.

The current could be established or interrupted either all at once or gradually. In the latter instance, the final gradient was reached in 10 seconds.

The experiments were conducted from January to June at laboratory temperatures (20° to 24° C).

1.2. Types of Reactions

The flow of current in the tank liquid produced reactions in the frog resulting in the following phenomena:

Variations in reflex activity: A light touch to the body generally induced a flight reflex. This reaction could be diminished with the animal apparently remaining insensitive, or else it could be enhanced with a simple bang on the table supporting the tank causing the hind legs to spring.

Muscle twitch: brief muscle contraction immediately followed by relaxation.

Tetanic contraction: muscle contraction resulting from the fusion of a series of twitches. Contraction was maintained for as long as the current flowed through the medium.

Rhythmic contraction: a succession of poorly fused and irregular twitches during the flow of current.

Paralysis: Absence of all muscle activity. The frog remained limp. (When placed on its back, it did not right itself until the current was switched off).

1.3. Classification of the Effects of the Current

1.3.1. With the Animal Kept Facing the Cathode

1.3.1.1. Effects of a Gradual Increase and Decrease in Current

a) With 0.2 to 0.4 volt per centimeter, there was an increase in reflex activity.

b) 0.3 to 0.5 volt per centimeter resulted in tetanic contraction of the hind leg muscles.

c) 1 to 2 volts per centimeter produced rhythmic contraction of a number of muscles in the body. This reaction was not abolished by prior curarization of the animal.

The animal quickly died if a gradient of this magnitude was maintained for over one minute.

1.3.1.2. Effects of a Sudden Transmission of Current

The sudden flow of current produced an additional reaction. The muscles in the hind legs twitched causing the animal to jump. This reaction was initiated with currents as low as 0.2 to 0.3 volt per centimeter. Interrupting the current rarely elicited a reaction.

1.3.2. With the Animal Kept Facing the Anode

1.3.2.1. Effects of a Gradual Increase and Decrease in Current

a) 0.3 to 0.6 volt per centimeter resulted in a gradual decrease in reflex activity. The animal was still capable of voluntary movement.

b) 0.4 to 0.8 volt per centimeter induced paralysis. The animal was incapable of righting itself when placed on its back. Complete relaxation of the muscles.

c) 1 to 2 volts per centimeter elicited rhythmic contraction of a number of muscles in the body. This reaction was not abolished by prior curarization of the animal. Currents producing this reaction quickly proved fatal.

1.3.2.2. Effects of a Sudden Transmission of Current

The sudden flow of current produced a further reaction. The hind leg muscles twitched causing the animal to jump. This reaction was observed with currents as low as 0.2 to 0.3 volt per centimeter. An identical twitch was produced when a flow of 0.8 to 1.0 volt per centimeter was interrupted.

1.3.3. With the Animal Perpendicular to the Flow of Current

1.3.3.1. Effects of a Gradual Increase and Decrease in Current

Beginning with a gradient of 0.3 to 0.5 V/cm, there was a tetanic contraction of the foreleg facing the anode. This leg was flexed under the animal's thorax, whereas the muscles in the other foreleg were relaxed. The muscles in the hind legs did not contract if the legs were kept perpendicular to the flow of the current.

1.3.3.2. Effects of a Sudden Transmission of Current

An additional effect was observed with the sudden flow of current. The muscles in the foreleg facing the cathode twitched. Generally, there was no reaction when the current was suddenly switched off.

2. - INTERPRETATION OF THE REACTIONS

As we have seen, the flow of direct current in the liquid medium surrounding the frog elicits a certain number of reactions in the animal. The reactions reflect either the current's excitatory effect (i.e., reinforcement of reflex responses; sustained muscle activity) or else, its inhibitory effect (i.e., depression of reflex responses, paralysis). Clearly, some of the current passes through the animal's body and exerts a direct effect on certain nerve or muscle components. In order to interpret the animal's reaction, it is important to have a thorough understanding of the effects of the current on the various excitable elements that might be involved.

With this objective, we carried out several simple experiments in which isolated nerve-muscle preparations of varying degrees of complexity were subjected to direct current. We were careful to conduct these experiments under physical conditions similar to those under which the reactions described in Section 1 were observed.

2.1. Characteristics of Contractions Resulting from Direct Excitation of Muscle Fibers

Our aim was to study contractile activity resulting from direct excitation of muscle fibers by direct current. Current was passed through the physiological solution to induce contractions in an isolated curarized muscle.

2.1.1. Experimental Procedure

The sartorial muscle was selected because of the regular arrangement of its fibers which lie parallel to one another.

The muscle was isolated and placed in a 10 X 4 cm tank made of insulating material and filled to a depth of 1 cm with Ringer's solution. The muscle was then curarized by adding gallamine triethiodide (Flaxedil, 5 mg per ml) to the solution.

One end of the muscle was fixed to the bottom of the tank by means of a needle through the partially preserved pelvic bone insertion, whereas the other end was attached to a mobile myograph lever with a strain gauge, connected to the first channel of a cathode-ray oscillograph.

The stimulating current was supplied by a storage battery connected to two silver chloride electrodes lining the inside of the ends of the tank. With a proper installation, it was possible to set up the current in one second and to maintain its intensity at a constant level.

The potential difference between the tank ends was recorded on the second channel of the oscillograph. By measuring this difference, it was possible to calculate the potential gradient (V/cm) resulting from the flow of current.

2.1.2. Results

Fig. 1 shows the contractile activity of the muscle for different potential gradients in the liquid medium. Reaction threshold values produced a simple twitch when the current was applied.

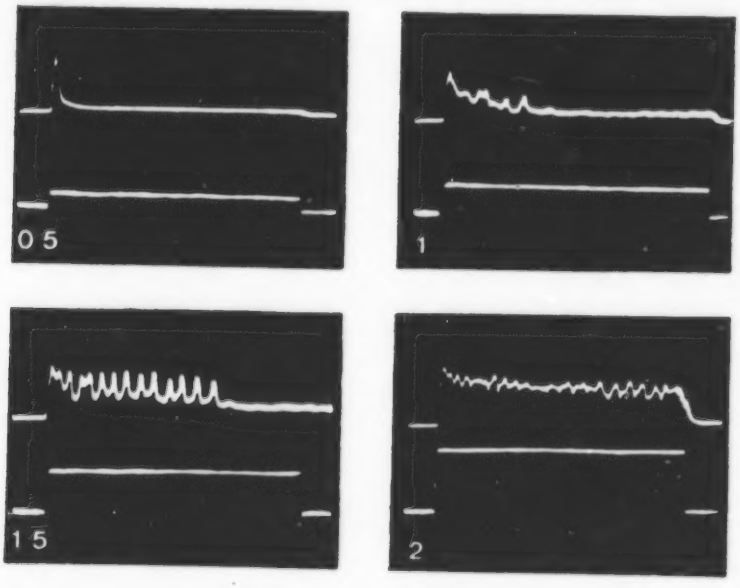

Fig. 1. Contractions of the curarized sartorial muscle in the frog induced by the flow of direct current in the physiological solution
(Potential gradients in V/cm; duration of current flow: 1 second).

Higher gradients (1 to 2 V/cm) resulted in a rhythmic succession of poorly fused muscle twitches. This activity persisted for as long as the current flowed. While the frequency of the twitches increased with the gradient, complete fusion did not occur.

If the position of the muscle was altered so that the muscle fibers were no longer strictly parallel to the flow of the current, the type of contraction remained unchanged, but a higher gradient was required to achieve this effect. The value was inversely proportional to the cosine of the angle of the muscle in relation to the flow of current. The relationship was similar to that established by Rushton (1927) for the nerve.

2.1.3. Discussion and Conclusions

Generally, even after curarization, frog skeletal muscles react to the flow of direct current in a physiological solution by a series of rhythmic contractions. The phenomenon occurs with a relatively high potential gradient, and the rate of repetition increases with the gradient (without, however, producing perfect fusion). When the gradient is relatively low, contractile activity may be reduced to a single twitch on application of the current. What we find here are the main characteristics of skeletal muscle fiber responses to galvanic current applied by means of large electrodes (Benoit and Benoit, 1936).

Stimulation of muscle fibers occurred under precisely the same conditions as when the current flowed through the body of the intact animal. Consequently, the characteristic rhythmic contractions recorded in animals subjected to high potential gradients (1 to 2 V/cm, described in sections 1.3.1.1.c and 1.3.2.1.c) were attributed to direct excitation of the muscle fibers.

2.2. Characteristics of Contractions Resulting from Excitation of Motor Nerve Fibers

Our next objective was to study muscle contractions that were indirectly induced by excitation of motor nerve fibers. For this purpose we used an isolated nerve-muscle preparation in which only the nerve fibers were subjected to the action of direct current.

2.2.1. Experimental Procedure

A nerve-muscle preparation consisting of the sciatic nerve and gastrocnemius muscle was placed in a two-compartment tank. A slot connected the two compartments (Fig. 2). The nerve was stretched parallel to the flow of the current in the compartment equipped with electrodes. The muscle, connected to the nerve, was placed through the slot into the other compartment. With this type of arrangement, the current flowing through the liquid affected only the portion of the nerve between the electrodes. Muscle contraction was recorded on a myograph fitted with a strain gauge. The potential difference at the ends of the nerve compartment and muscle contraction were simultaneously recorded on both channels of the cathode-ray oscillograph.

2.2.2. Results

Fig. 2 shows the contractions obtained when currents of different intensities were abruptly passed in each direction (ascending or descending current for the nerve) and suddenly cut off. The ascending current values given are those which produced contractions of essentially the same amplitude as recorded with the descending current. The short duration of the muscle contractions indicates that excitation of the nerve fibers occurred with the onset of flow of low-intensity currents and the onset and cessation of flow of

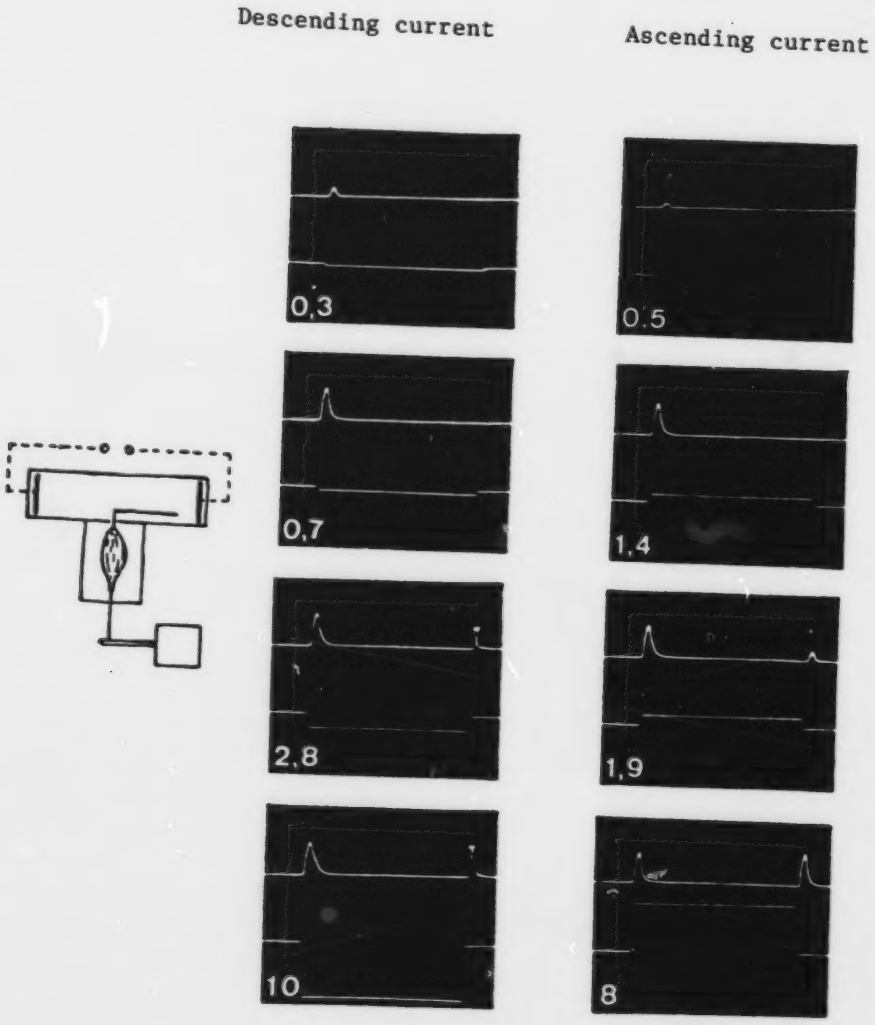

Fig. 2. Contractions of the gastrocnemius muscle in the frog produced by the flow of direct current in the physiological solution bathing the sciatic nerve.
(potential gradients created in the solution in the nerve compartment in V/cm; duration of current flow: 1 second).

strong currents. There was no activity, however, throughout the flow of current. It will be noticed, however, that when the descending current was applied, the duration of the contraction increased slightly with the intensity of the current.

When the nerve was positioned in the tank so that the nerve fibers were no longer strictly parallel to the flow of current, there was no change in the type of contraction, but stronger currents were needed to elicit a response. The reaction threshold was inversely proportional to the cosine of the angle of the nerve in relation to the flow of current. This relationship concurs with the findings published by Rushton (1927).

When the current flowing through the compartment was progressively applied and decreased, no contraction occurred.

2.2.3. Discussion

When direct current flows through a volume of liquid bathing frog motor nerve fibers, excitation of the latter follows the same basic pattern described by Pflüger (1859) for a nerve placed on two electrodes. While the order of occurrence and increased amplitude of muscle responses when the current is applied and interrrupted are similar to those reported by Plüger, we failed to observe any "shutdown" response to high-intensity ascending current in our experiments. In all likelihood, the density of current entering the nerve fibers is much weaker in a uniform electric field than at an electrode placed on the nerve, and anelectrotonus, which blocks conduction, is more difficult to achieve.

The short duration of the contraction on application of the current indicates there is no repetition of response by the nerve fibers throughout the duration of current flow. The progressively longer duration of the contraction, however, as the intensity of the descending current increases suggests rapidly damped rhythmic activity in certain motor units. By placing recor[illegible]g electrodes on the portion of the nerve penetrating the side compartment, it could be demonstrated that some nerve fibers were the site of activity of this type for several tenths of a second after the current was applied. These damped rhythmic responses triggered by the passage of direct current are similar to those described by Fessard (1936) in the frog sciatic nerve.

2.2.4. Conclusions

Excitation of motor nerve fibers by the flow of direct current through an animal occurs only if the current is abruptly applied or shut off. It results in a brief contraction of the effector muscles at the time the current is established or interrupted. These characteristics correspond to those of reactions described in sections 1.3.1.2. and 1.3.2.2. We consequently attribute these reactions to excitation of certain motor nerve fibers by the passage of current through the animal's body.

2.3. Characteristics of Contractions Resulting from Motoneuron Excitation

Muscle contractions arising from the passage of current through the animal's body may also result from excitation of spinal motoneurons. In order to test this hypothesis, we sent currents through the bodies of frogs in which the brains had been destroyed. The direction and intensity of the currents were regulated and in each specimen, the resulting muscle activity in the gastrocnemius muscle of one of the two hind legs was recorded.

In order to interpret the results obtained in these experiments, it must be remembered that in the frog, the sciatic nerve fibers and lumbar roots lie largely parallel to the axis of the body. Because of this orientation, they are readily excited by an electric current flowing lengthwise through the animal. As we have shown above, excitation is produced when the current is abruptly applied and at times, suddenly interrupted, causing twitching of all hind leg muscles. In our experiments, it may be assumed that these twitches occur in addition to whatever muscle activity might result from excitation of the motoneurons. Because of the origin of these twitches, they should occur only when the current flowing through the animal's body is suddenly applied and interrupted. They should not disappear on destruction of the spinal cord.

2.3.1. Experimental Procedure

As in the procedure described in Section 2.1.1., the stimulating current was supplied by a storage battery. The intensity was regulated and kept constant by electronic means.

A decerebrated frog was fixed to a board and the gastrocnemius muscle of the right leg, isolated. The distal end was attached to a myograph with a strain gauge. The myogram was recorded on the first channel of a cathode-ray oscillograph and the stimulating current on the second.

The left sciatic nerve was sectioned so as to prevent excitation of the fibers of this nerve by the polarization current.

In most of the experiments, the current passed through the body, between the two electrodes. One electrode was placed in the mouth, and the other,

sling-shaped, around the left hind leg.

These electrodes consisted of a silver chloride plate and a sheath of cotton soaked with physiological solution. Because of their large size and chlorination treatment, relatively intense currents could pass through the preparation for several seconds without any risk of polarization.

In some of our experiments, for control purposes, we used another type of electrode designed to send most of the current through the spinal canal. In this instance, the cephalic electrode consisted of a large glass pipette filled with Ringer's solution. A long coiled silver chloride wire was introduced into the pipette. The narrow end of the latter was applied between the skull and the first vertebra after part of the articular ligaments had been sectioned.

The results obtained with both types of electrodes were comparable in every respect.

2.3.2. Results

Fig. 3 shows the different types of contraction achieved in the gastrocnemius muscle when an electric current of adequate intensity flowed through the body of the animal. The two columns of myographs correspond to the two directions of the flow, i.e., a postero-anterior flow to the left with the cathode at the head and anteroposterior flow to the right with the anode at the head. Each horizontal row correponds to a given current intensity, expressed in milliamperes.

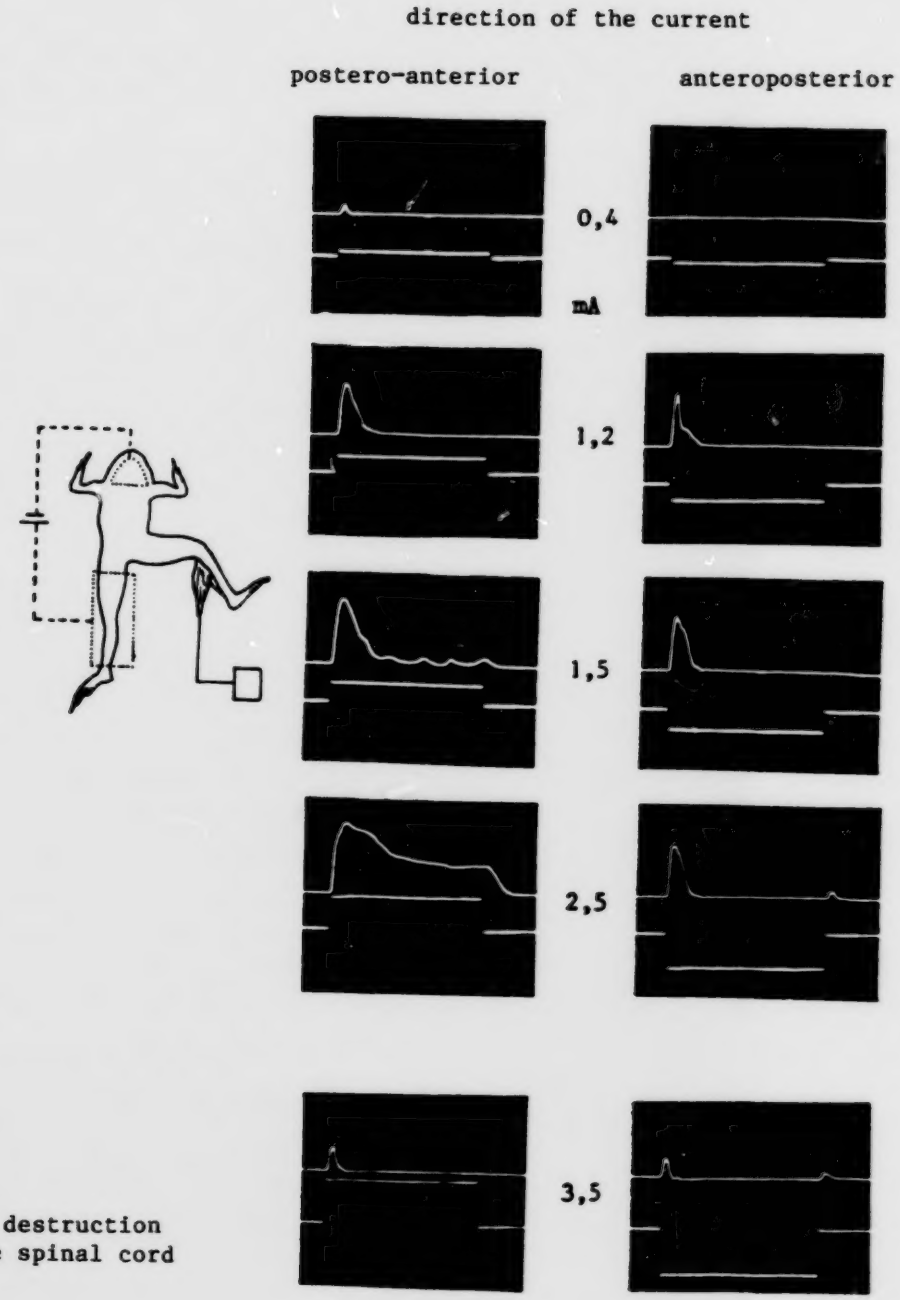

Fig. 3. Contractions of the gastrocnemius muscle in the frog induced by the passage of direct current through the body of a specimen in which the brain was destroyed.
(potential gradients in V/cm; duration of current: 1 second).

The bottom row of recordings was obtained after destruction of the spinal cord by means of a long needle inserted deep into the spinal canal.

Contractions of the gastrocnemius muscle differed with the direction of flow as well as the intensity of the current. We observed:

- twitches which occurred when the current was abruptly applied and interruped. They largely persisted after destruction of the spinal cord;
- tetanic activity which persisted for as long as the current flowed through the body. This activity occurred only with postero-anterior currents of adequate intensity and increased with the intensity of these currents. It disappeared completely after destruction of the spinal cord.

In these experiments, only contraction of the gastrocnemius muscle was recorded. It was observed, however, that all the muscles in the right hind leg (the sciatic nerve had been left intact) exhibited the same contraction patterns.

2.3.3. Discussion

2.3.3.1. On the Origin of Tetanic Activity

After destruction of the spinal cord, the flow of direct current postero-anteriorly no longer caused tetanic contraction of the still functional hind leg. It is very likely, therefore, that the tetanic activity observed was due to motoneuron excitation.

Barron and Matthews (1938), Alanis (1953) and Fuortes (1954) demonstrated that direct current could trigger rhythmic activity in motoneurons. For this to occur, the current must flow from the ventral root to the spinal cord. In this case, the current "exits" at the cell body and the proximal region of

the axon (intermediate segment). As a result, it tends to depolarize these structures. When there is a sufficient degree of depolarization, the motoneuron enters into a state of rhythmic activity, which it maintains since it is slow to accommodate (Frank and Fuortes, 1960).

Granit, Kernel and Shortess (1963) demonstrated by means of transmembrane stimulation that the frequency of the action potentials produced by the motoneuron increased with the intensity of the exiting stimulating current.

These findings from the literature corroborate our own findings:

- tetany in the hind leg muscles occurred only when the direction of flow made the current exit at the corresponding motoneuron cell bodies (under the experimental conditions of Fig. 3 as well as those of the in vivo trials in Section 1.3.1);

- tetany in the foreleg muscles (1.3.3.1.) was achieved under the same conditions when the animal in the tank was placed perpendicular to the flow of current. In this case, only the muscles of the leg on the side of the anode contracted;

- the intensity of tetanic contraction depended on the intensity of the current, suggesting not only that new motor units came into play, but also that rate of response of each motoneuron was greater.

2.3.3.2. On the Twitches Occurring with Application and Interruption of the Current

The twitches produced when the current flowing through the animal was established and interrupted can result only from direct excitation of sciatic motor nerve fibers innervating the gastrocnemius muscle. They cannot be due to excitation of the motoneurons since they persist after the spinal cord has

been destroyed. Nor can they arise from direct excitation of the gastrocnemius muscle since, under the experimental conditions we adopted (Fig. 3), it lay outside the flow of current. The sciatic motor nerve fibers, which are parallel to the body's axis over much of their length, however, can easily be activated on the sudden application and interruption of current and cause muscle twitches in the lower limbs (Section 2.2.2). It is therefore normal for these twitches to occur according to the pattern described by Pflüger (1859).

Since these twitches are not of central origin, it may seem surprising that the amplitude and duration decrease following destruction of the spinal cord (Fig. 3).

Two hypotheses are possible,i.e., the current directly stimulates certain descending fibers of the spinal cord and/or the sensory fibers in the sciatic nerve. The conditions for excitation by the current flowing through the animal are as favourable for these sensory fibers as for the motor fibers. As long as the spinal cord remains intact, excitation of some of these sensory fibers should, by reflex action, cause contraction of the gastrocnemius muscle. This contraction then in turn extends the twitch response resulting from excitation of the motor nerve fibers. After destruction of the spinal cord, muscle activity is reduced because the contraction reflex no longer takes part in muscle activity.

2.3.4. Conclusions

As we have just seen, the flow of direct current through a frog is capable of triggering rhythmic activity in motoneurons traversed by the current in an axon-soma direction.

We consider the reactions described in Sections 1.3.1.1. and 1.3.3.1 the result of an effect of this type.

2.4. Current-Induced Modification of Reflex Activity and Paralysis

Changes in the animal's reflex activity was one of the current-induced effects described in Section 1.3. It was found that a postero-anterior current heightened the response to an external stimulus (causing the animal to jump at the slightest vibration of the table), whereas an anteroposterior current reduced reflex (or voluntary) activity causing real paralysis when delivered at high intensities. We set out to provide a clear demonstration of this moderating effect and tried to explain it.

Two methods were used to study modifications in spinal reflex activity under the influence of direct current flowing longitudinally through the animal. In a first series of experiments, we recorded the reflex contractions of the semitendinous muscles of one of the hind legs initiated by application of a brief electric shock of constant intensity between two toes of this same leg. In a second series of experiments, we recorded the response of ventral-root nerve fibers to a short stimulus of contant intensity, applied to the corresponding dorsal root.

2.4.1. Modification of the Reflex Contraction of the Semitendinous Muscle

2.4.1. Experimental Procedure

Fig. 4 indicates the direction of flow and intensity of the current to which the frog was subjected.

The direct current to be sent through the animal's body was produced and applied as described in Section 2.3.1. For the reasons discussed earlier

(2.3.1.), the sciatic nerve of the left leg was sectioned at the level of its lumbar roots.

Two plastic fingers filled with physiological serum and containing tiny silver chloride electrodes at the deep ends were placed on the second and third toes of the right leg and connected to a stimulator discharging brief electric shocks of a few volts.

The distal end of the semitendinous muscle which takes part in the leg's withdrawal reflex was isolated and connected to a myograph with a strain gauge. The myogram and deflection signalling application of the stimulus to the toes were simultaneously recorded, making it later possible to measure the latency period for the reflex response.

In order to obtain reflex contractions of the same amplitude, in the absence of a polarizing current, it was absolutely necessary for the stimuli to be regularly applied (the preparation had to be stimulated even if records were not made) at a very low rate of one stimulus per minute. These conditions were met throughout the experiment.

2.4.1.2. Results

Myograms of reflex contractions of the semitendinous muscle in response to a brief electric shock applied between the toes of the same leg are shown in Fig. 4. Columns 1 and 4 contain control myograms recorded before and after the passage of current through the animal's body. The myograms in column 2 were made during the postero-anterior flow of current and those in column 3, during the anteroposterior flow of current. Each row represents a different intensity of current, expressed in milliamperes.

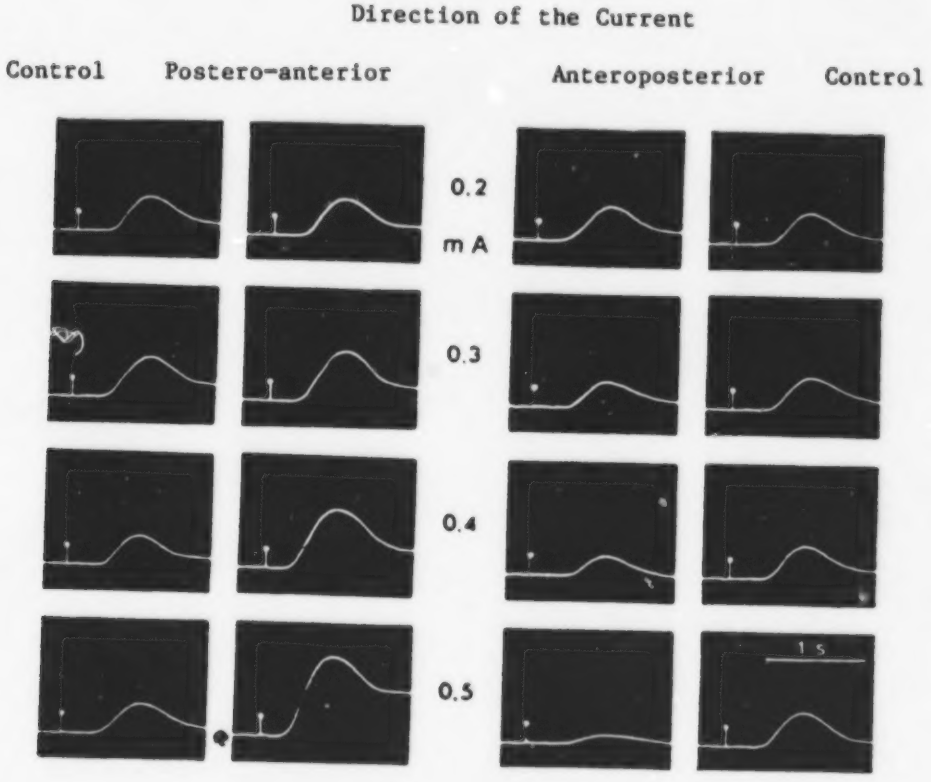

Fig. 4. Reflex contractions of the semitendinous muscle in the frog in response to a brief electric stimulus applied to the toes on the same leg. Effect of direct durrent flowing through the body of the animal.
(intensity indicated in milliamperes).

It may be noted that all throughout the experiment, the amplitude of the control reflex contractions remained essentially the same. When a low-intensity postero-anterior current flowed through the animal's body, however, the amplitude and duration of the reflex contraction increased. In the case of the anteroposterior flow of current, on the other hand, the contraction was reduced. Increases in current intensity were attended by a corresponding reinforcement and dampening of the reflex response.

The signal preceding the myogram indicates the precise moment when the stimulus was applied to the animal's toes. The latency period for each reflex response could then be assessed for each record. The latency period was shorter when the current flowed in a postero-anterior direction. Conversely, it was longer when the current flowed in the opposite direction.

Fig. 4 shows only the results obtained with polarizing currents of less than 5 mA, since the range provided was broad enough to allow us to explain the reactions observed in Sections 1.3.1.1.a and 1.3.2.1.a and b.

Apparently with higher intensities, the amplitude of the reflex contraction decreases with posterio-anterior current, but remains unchanged with anteroposterior current.

An interpretation of this phenomenon would require further, more detailed experimentation but the simplest hypothesis is that intense currents are capable of creating blockages at certain points of the reflex circuit. Such blockages are similar to those observed in cathode depression.

2.4.2. Modification of the Reflex Discharge Recorded at the Ventral Root

In this series of experiments, our aim was to show how motoneuron activity was altered by the passage of direct current through the animal's body.

2.4.2.1. Experimental Procedure

A spinal frog was prepared by destroying the brain. The 8th, 9th and 10th spinal nerves were isolated by laminectomy and treated with paraffin oil to protect them from drying. On the animal's right side, the dorsal root of the 10th nerve was slightly raised by means of two small platinum hooks acting as stimulation electrodes, whereas two other platinum hooks were placed in contact with the corresponding ventral root and served as reception electrodes. The proximal electrodes in each pair were 8 to 10 mm from the spinal cord.

On the left side of the animal, the sciatic nerve was sectioned in the abdominal region.

A brief stimulus, i.e., 0.1 ms, was applied very regularly (every minute) to the dorsal root by an electronic stimulator fitted with an HF insulation unit. The reflex electrical activity occurring at the ventral root was recorded by means of a cathode-ray oscillograph. The direct current sent through the animal's body was produced and applied in accordance with the procedure outlined above.

2.4.2.2. Results

Fig. 5, at the top, shows a non-maximum reflex response produced and recorded under the conditions just described. It appeared after a latency

period of several milliseconds as a succession of non-synchronized action potentials occurring over a 10-to-20-millisecond period.

The reflex response was altered when a low-intensity current was sent through the animal's body. A 1 mA postero-anterior current reinforced the response, whereas an anteroposterior current of the same intensity abolished it completely. There was less response reinforcement or depression with lower intensity currents.

2.4.3. Discussion

Like the electrical tracings at the ventral roots, the myograms showed that reflex activity could be modulated by the passage of direct current through the animal's body. The phenomenon was demonstrated earlier by Bremer (1941), Bremer and Bonnet (1942) and Bonnet (1956) under different experimental conditions. A certain number of arguments suggest that this modulation is due to a direct action by the current on the spinal motoneurons.

Thus, the current's modulating effect seems to be independent of the complexity of the pathways excited by the reflex-inducing stimulus. In Bremer's experiments in cats (1941), the experimental conditions and choice of animal made it possible to demonstrate the effects of polarizing current when monosynaptic pathways were involved. In Bonnet's experiments (1956) as well as our own in which frogs were used, the modulating effect of the current was observed during activation of polysynaptic pathways (In frogs, monosynaptic pathways cannot be excited by a single reflex-inducing stimulus). It is therefore very likely that polarizing current acts primarily on the motoneurons.

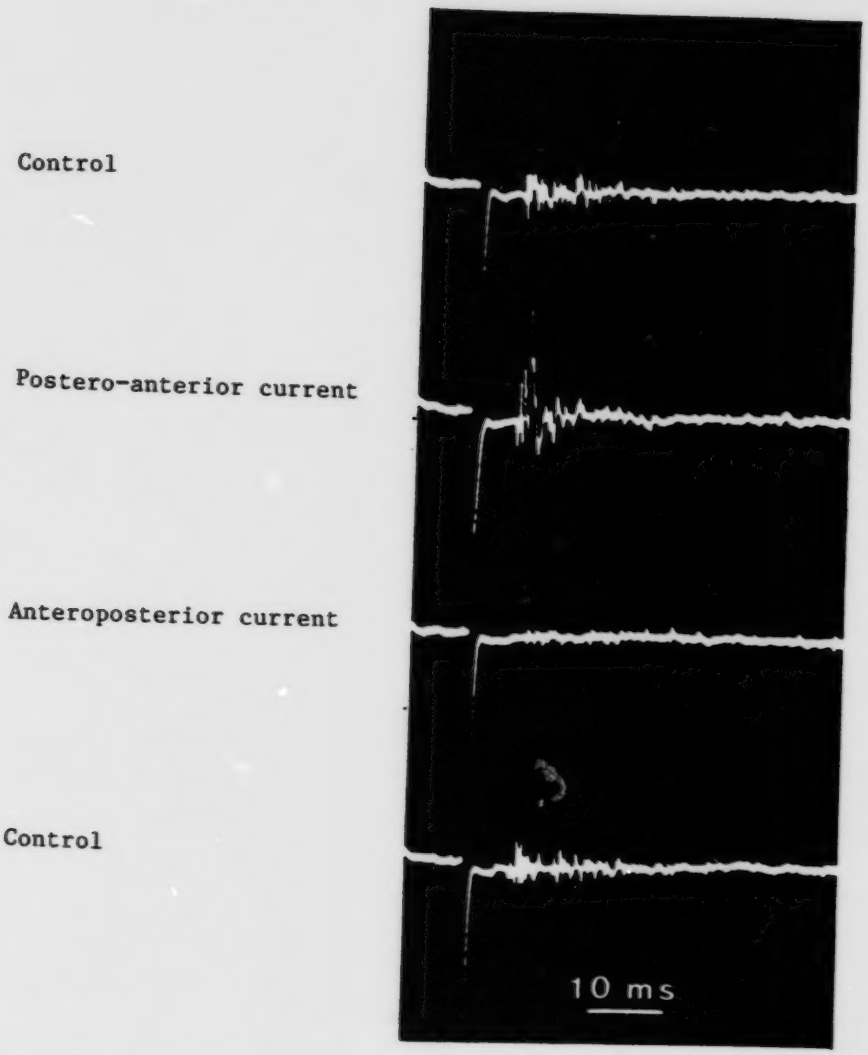

Fig. 5. Electrical activity at the ventral root of the 10th nerve in the frog in response to a brief stimulus applied to the corresponding dorsal root.
Effect of a 1 mA direct current flowing through the animal's body.

It was also observed in free-swimming animals as well as in the experiments described in this section that reinforcement of reflex activity and tetany were produced by currents flowing in the same direction, differing only in intensity. In the discussion in the previous sections, tetany was attributed to current in which the direction of flow and intensity were suitable for depolarizing certain neurons, setting up rythmic activity in these neurons. It is therefore logical to assume that while a lower-intensity current flowing in the same direction produces depolarization of these same motoneurons, the amplitude is smaller and inadequate to trigger rhythmic activity. It is sufficiently great, however, to heighten motoneuron excitability by a considerable degree. By this action, the current passing though the animal's body increases the number of motoneurons excited by afferent stimulation. This is illustrated in line 2 of Fig. 5 where there was an increase in amplitude in the overall responses recorded at the ventral root.

The depression of reflex activity and paralysis obtained by an anteroposteror current may be similarly explained. Since nerve cell bodies are depolarized when current exits at that level, resulting in enhanced reflex activity, it may be assumed that hyperpolarization is produced by an entering current.

By this action, the current flowing through the animal's body reduces the number of motoneurons that can be excited by afferent stimulation. In the experiment in Fig. 5, the intensity of the current was adequate to suppress all reflex activity (line 3). Under these conditions, it is also likely that any voluntary activity would have also been impossible. The current caused paralysis by hyperpolarization of the motoneurons.

Another hypothesis is that an anteroposterior current reduces reflex activity by blocking conduction in certain sensory nerve fibers. Because of the anatomical arrangement of the lumbar nerves, afferent impulses are propagated in a direction opposite to that of the polarizing current. They consequently travel toward areas in a state of anelectrotonus. This could be locally reinforced by an anatomical arrangement favouring some concentration of the electric flow reaching the nerve fibers.

This hypothesis was discarded after the following finding was made. When the dorsal root is sectioned a few millimeters from the spinal cord and, with the aid of excitation electrodes, held in the oil covering the operative field, the afferent nerve fibers are no longer traversed by the current flowing through the animal, at least not in their extra-spinal portion. Despite the electrical insulation of the afferent fibers, reflex activity may still be inhibited by the passage of direct current. The anteroposterior currents must therefore exert their basic depressant effect at the level of the motoneurons.

2.4.4. Conclusions

We must therefore conclude that modifications of reflex activity arising from the passage of current through the animal's body result from the same basic effect, i.e., changes in the nerve membrane potentials by the current flowing through the membranes.

The direction of the current therefore determines the effect produced, i.e., excitation or depression. Electrical stimulation of motoneurons in an axon-cell body direction causes heightened activity (amplified reflex, tetany),

whereas stimulation in a cell body-axon direction depresses activity (diminished reflex, paralysis).

The current can produce its effect only if it encounters properly oriented neurons on its path. The most favourable orientation is parallel to the flow of current, while the least favourable is perpendicular to the flow. When a current travels longitudinally through the body of a frog, it is the operation of the hind legs that is primarily affected since the motor neurons of the sciatic nerves lie parallel to the axis of the animal's body. On the other hand, this current has very little effect on the operation of the forelimbs as the motor neurons of the brachial nerves lie largely perpendicular to the body axis.

These phenomena would explain the reactions described in 1.3.1.1 a and 1.3.2.1. a (heightened and diminished reflex activity in the frog).

3. - GENERAL CONCLUSIONS

When an aquatic animal is placed in a liquid medium traversed by direct current, it exhibits a certain number of motor disorders which hinge on the position of the animal in relation to the flow and intensity of the current. In the course of our experiments with frogs, it was found that each reaction had its own characteristics. Furthermore, the reactions could be repeated whenever the parameters involving the orientation and intensity of the current flowing through the animal were reproduced. By isolating some neuromuscular structures of varying degrees of complexity and subjecting them to the current under conditions which most satisfactorily reproduced <u>in vivo</u> conditions, it was possible to single out the structures which, when directly excited by the current, set off the motor reactions observed in the intact animal.

The effects of the current fall into two categories:

- excitatory effects (muscle twitches, tetanic muscle contractions, heightened reflex responses) produced when the physical conditions of the flow of current are favourable to direct depolarization of the excitable elements involved in these reactions (muscle fibers, nerve fibers, motoneurons, respectively).

- inhibitory effects (reduced reflex responses, paralysis) when the conditions of the flow of current favour direct hyperpolarization of these elements (motoneurons).

The wide range of responses attending the flow of current through an animal's body may therefore all derive from the same fundamental process, i.e., a change in the membrane potential of excitable elements that are favourably oriented in relation to the flow of current. The excitatory effects of direct current are thought to differ with the types of responses that are specific to the depolarized membranes (activity with a high repetition rate in the case of motoneurons, with a low repetition rate in the case of muscle fibers and non-repeated activity in the case of motor nerve fibers). This is a logical hypothesis considering the electrotonus-producing effects of direct current that have been demonstrated in numerous studies and our own findings. We have shown, in particular, that modulation of reflex activity in relation to the direction of flow of the current passing through the animal's body seems to be linked to a variation in motoneuron excitability. This can only be due to changes in the membrane potential of the motorneurons under the effect of the current. We are currently involved in experimentally demonstrating the phenomenon by directly recording the transmembrane potential of certain motoeurons.

In the present study, we have focused our attention on the effects produced by current on certain motor structures (motoneurons, motor nerve fibers, muscle fibers). Despite these constraints, it was possible to explain all of the reactions recorded. It is likely, however, that the current flowing through the animal also acts on certain sensory structures (receptors and sensory nerve fibers). While an action of this type does not seem to play a fundamental role in the reactions of the frog, it may be more important in the reactions of other aquatic animals. A study of the effect of direct current on sensory structures will provide useful information.

Likewise, the excitation and inhibition phenomena we described for motoneurons should also apply to associative structures. This means that the organization of motor activity could be disturbed by the passage of current through an animal's body. In actual fact, it does not seem to be possible to demonstrate galvanotaxis in frogs. In other aquatic animals, particularly in fish, direct current can induce a swimming pattern in which the animals systematically face the same electrode.

The conclusions we have drawn from our work with frogs can probably be transposed to certain reactions in other aquatic species; namely, fish (Blancheteau et al., 1961). Thus, for instance, exacerbated reflexes and tetany of the nerves induced by the passage of a direct current postero-anteriorly, galvanic paralysis produced by an anteroposterior current, and anode flexure resulting from a transverse current appear to correspond to the reactions we have described and explained in frogs. There are a certain number of fish-specific phenomena, however, that could not be studied, i.e., anode galvanotaxis, in particular, which does not appear to occur in adult

adult frogs. We intend to study these phenomena, applying the methods that helped to further our understanding of the reactions occurring in the frog.

Received for publication in March 1971.

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